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Multiresidue pesticide quantitation in multiple fruit matrices via automated coated blade spray and liquid chromatography coupled to triple quadrupole mass spectrometry

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ABSTRACT

Application of ambient mass spectrometry techniques to accelerate analysis of pesticides in produce, with technique validation via chromatographic separation, has not been explored extensively. In this work, coated blade spray (CBS) was used to provide freedom of instrumental choice for a multiresidue panel of pesticides in apple, blueberry, grape, and strawberry through direct-coupling with mass spectrometry (MS) and liquid chromatographic (LC) analyses. For all four matrices, > 125 compounds were found to meet European Union guidelines concerning linearity, precision, and accuracy while both CBS-MS/MS and SPME-LC-MS/MS methods achieved limits of quantitation below their minimum regulatory limits. Additionally, results for samples containing residues (n = 57) yielded good agreement between instrumental methods (percent differences < 20% for 73% residues), supporting CBS as a stand-alone technique or complement to LC confirmation of pesticides in fruit matrices.

1. Introduction

Different global pesticide regulatory limits and legislation, coupled with increasingly globalized trade, can result in the import of products containing significant pesticide residues (Galt, 2008; Neff et al., 2012). Seasonality, produce-focused dietary trends, and climate restrictions mean many countries rely on imported fruit to meet demand. As a side-effect, pesticide residue regulations involve extensive product screening programmes, resulting in hundreds of thousands of samples annually in tens of matrices for hundreds of pesticide products and additives (Agricultural Marketing Service, 2019; EFSA (European Food Safety Authority), 2018). Gold-standard sample preparation for such analyses involve some form of homogenization often followed by a workflow based on quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003), which has been shown to be broadly applicable to many food matrices, with only minor modifications, low cost, low environmental impact, and generate cleaner final extracts compared to solvent extraction protocols (SE) (Santana-Mayor, Socas-Rodríguez, Herrera-Herrera, & Rodríguez-Delgado, 2019; Zhang et al., 2011). However, QuEChERS suffers from significant sample, standard, and solvent usage, which result in non-trivial automation—a compromise for sample preparation prior to chromatographic analysis.

In contrast, the advent of ambient mass spectrometry (AMS) and the pledges of sample preparation-less and separation-free analysis methods have resulted in promising applications of pesticide screening in produce matrices—be it on-site or in-lab (Lu et al., 2018). Desorption electrospray ionization (DESI) and paper spray (PS) are two of such AMS electrospray-based techniques for which an assortment of applications has been described, with some spilling into food analysis. PS has been demonstrated as a pesticide screening tool for homogenized samples diluted with organic solvents and with produce peel wiping as a sampling method (Evard, Kruve, Lõhmus, & Leito, 2015; Moura et al., 2020). Spotting produce homogenate on PS cartridges was found to reduce spray reproducibility, and wiping protocols suffer from quantitation limitations, resulting in cumbersome coupling of sample preparation to screening with PS and further confirmation with LC. Similarly, DESI analysis of untreated peel and homogenate samples analyses have encountered ionization suppression with non-trivial internal standard application limiting quantitation efforts (Gerbig et al., 2017; Mainero Rocca, Cecca, L'Episcopo, & Fabrizi, 2017). These difficulties warrant the investigation of hybrid methodologies that provide integrated sample preparation, the speed and simplicity of AMS, and ease of coupling to separation techniques for more robust analysis.

One of such techniques is coated blade spray (CBS)—an integrated sampling, sample preparation, and sample introduction device

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composed of a polymeric sorbent coated on a conductive support (Gómez-Ríos & Pawliszyn, 2014). The geometry allows for the preconcentration of analytes via solid-phase microextraction (SPME) followed by on-line desorption and voltage application resulting in electrospray (ESI). CBS has demonstrated savings in analysis time, sample use, and solvent use with quantitation of pharmaceuticals in biofluids, wastewater, and multiresidue pesticide analysis in fruit juice (Gómez-Ríos et al., 2018; Kasperkiewicz, Gómez-Ríos, Hein, & Pawliszyn, 2019; Poole, Gómez-Ríos, Boyaci, Reyes-Garcés, & Pawliszyn, 2017). Following extraction, the SPME substrate can be coupled to a mass spectrometer or desorbed into a vessel for additional sample characterization or validation via chromatographic analysis, as demonstrated in previous SPME food analysis applications (Gómez-Ríos, Gionfriddo, Poole, & Pawliszyn, 2017; Khaled, Gionfriddo, Acquaro, Singh, & Pawliszyn, 2019; Souza-Silva & Pawliszyn, 2015; Xu et al., 2016). Food control workflows involve screening of samples for contaminants of interest followed by confirmation and quantitation of any suspected samples from the screening workflow. Given the substantial sample-load that regulatory agencies encounter, the incorporation of an AMS screening or quantitation tool to reduce analysis time, cost, and improve scalability while also remaining compatible with follow-up sample confirmation via LC-MS can prove beneficial (Blokland, Gerssen, Zoontjes, Pawliszyn, & Nielen, 2020).

Concisely, this work presents a workflow (as shown in Fig. 1) for the multiresidue (*e.g.* organophosphates, organonitrogen, carbamates, neonicotinoids, strobilurins, triazines, spinosyns) quantitative analysis of 126 pesticides in apples, 139 pesticides in blueberries, 136 pesticides in grapes, and 135 pesticides in strawberries via CBS-MS/MS and SPME-LC-MS/MS with a comparison of analytical figures of merit (*e.g.* linearity, accuracy, precision, limits of quantification [LOQ]), analysis properties (*e.g.* solvent usage, analysis time), and real-world sample quantification and comparison between techniques. The comparable results between methodologies (*i.e.* real-world sample percent difference < 30% and similar figures of merit) support CBS-MS/MS as a rapid quantification tool for pesticides in the fruit matrices investigated as either a stand-alone workflow or as an *a priori* complement to LC-MS/MS validation.

2. Materials and methods

2.1. Chemicals and sample preparation devices

LC/MS grade methanol (MeOH), acetonitrile (ACN), and water were acquired from Fischer Scientific (Hampton, NJ, USA). LC/MS grade formic acid was acquired from Sigma Aldrich (St. Louis, MO, USA). LC/MS grade ammonium formate and ammonium acetate were acquired from Sigma Aldrich. Sodium acetate and acetic acid were acquired from Sigma Aldrich. Pesticide standards, acquired and used as part of a series of mixtures, totaling 204 compounds (LC Multiresidue Pesticide Kit), were provided by Restek Corporation (Bellefonte, PA, USA). Deuterated analogues used as internal standards (atrazine-d_5, carbofuran-d_3, cyprodinil-d_5, dimethoate-d_6, imazalil-d_5, kresoxim-methyl-d_7, malathion-d_6, metalaxyl-d_5, methiocarb-d_3, oxamyl-d_6, spirotetramat-d_5, trifloxystrobin-d_6, fludioxonil-$^{13}C_2$) were acquired from Toronto Research Chemicals (Toronto, ON, CA). All standards were stored at original equipment manufacturer (OEM) concentrations (1000 or 100 $\mu g \cdot mL^{-1}$ in MeOH or ACN) at −80 °C. The stainless-steel blades used for the manufacture of the CBS devices were purchased from Shimifrez Inc. (Concord, ON, CAN). The 5 μm hydrophilic lipophilic balance (HLB) particles were synthesized in-house and have been

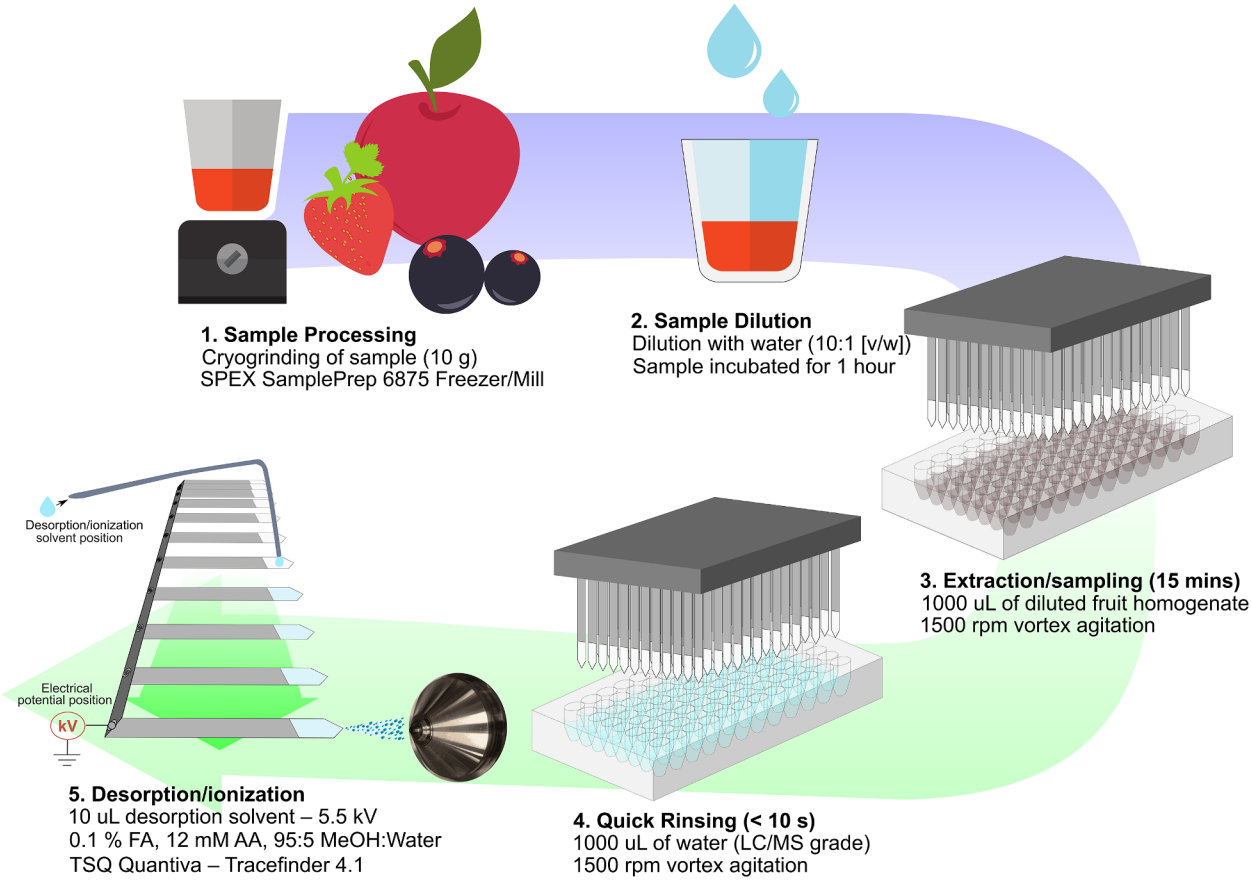

Fig. 1. Demonstrated workflow for the analysis of pesticides in fruit matrices (steps 1 - 4) for CBS-MS/MS (step 5). The LC-MS/MS analysis method employed the same sample preparation workflow (steps 1 - 4).

characterized and described in detail elsewhere (Khaled et al., 2019). Blades consisted of a coating length of 10 mm were prepared and dip-coated with HLB particles via a procedure developed in-house, described elsewhere (Lendor, Gómez-Ríos, Boyacl, Vander Heide, & Pawliszyn, 2019).

2.2. Sample preparation

2.2.1. Fruit sample processing

Blank matrices (apples, blueberries, strawberries, and grapes) used for matrix-match calibration and validation were sourced from local grocery markets of the organic variety, with > 400 g of each matrix purchased. Real-world samples were purchased from July–August 2019 from local grocery markets with species, country of origin, and purchase data recorded (Table S4; 3 apple samples, 3 blueberry samples, 5 grape samples, 4 strawberry samples). Matrices were cryoground in batches of 20 g each, pooled and stored according to matrix type, using a liquid nitrogen bath cryogrinder (6875 Freezer/Mill from SPEX SamplePrep, Metuchen, NJ, USA) until a fine powder consistency was observed (approximately 3 min). Ground matrices were stored in glass at −80 °C until use. The same protocol was used for the preparation of real-world samples. Grinding vessels were cleaned thoroughly with detergent, water, and methanol between samples.

2.2.2. Analytical procedures for optimization experiments, validation, and real samples

Standard spiking was completed by mass, with initial method optimization experiments utilizing batches of 10 ± 0.05 g of each matrix. A dilution level investigation was carried out by spiking 1.0 g, 1.5 g, 3 g, and 5 g of fruit homogenate with 50 ng/g of the pesticide mixture and subsequently diluting these mixtures with 9 mL, 6 mL, and 3 mL of water to yield 0.1, 0.2, 0.5, and 1 dilution levels, respectively. Final method validation was performed using spiked 1 ± 0.01 g aliquots of matrix per concentration level (*i.e.* calibration point, validation point, real-world sample) diluted in 9 mL water. IS were spiked at the 20 ng/g level for calibration and validation experiments. Calibration and validation working standards were spiked into the sample at the required concentration levels, with the undiluted fruit homogenate spiked not to exceed the addition of > 2% organic solvent content (pre-dilution). Before extraction, spiked samples were incubated at 4 °C for 12 h to allow for equilibration of pesticides within the sample. Extractions were performed from 1 mL sample volume (corresponding to 0.1 g of fruit homogenate per sample) from all matrices at room temperature, with agitation (approximately 1200 rpm) for 15 min. A coating washing step in water of 10 s was implemented to remove matrix particulate from extracted blades prior to desorption for CBS-MS/MS or LC-MS/MS analysis.

3. Method validation

3.1. Instrumental parameters

All experiments described were completed using a TSQ Quantiva from Thermo Scientific (San Jose, CA, USA), with data analysis completed using Trace Finder 4.1 from Thermo Scientific. Positive ionization mode coupled with single-reaction monitoring (SRM) mode was used for all analyses, and MS/MS compound transitions and conditions (Table S1) were optimized via direct infusion from methanolic standards.

3.1.1. CBS-MS/MS analysis

All manual CBS desorption and ionization experiments were performed using a custom CBS source built at the University of Waterloo, which is described elsewhere (Tascon et al., 2017). Automated CBS analysis was performed using an autosampler made by Professional Analytical Systems Technology and modified in-house; with development and validation described elsewhere (Kasperkiewicz et al., 2019). The desorption solution used was 95:5 MeOH/water v/v, 0.1% formic acid, and 12 mM ammonium acetate. All experiments utilized 10 μL of the desorption solution. A desorption time of 12 s was used for both manual experiments and autosampler experiments. Upon analyte desorption, 5.5 kV voltage was applied for 10 s, resulting in the ionization and introduction of analytes to the MS entrance via the electrospray generated at the tip of the blade. A spray time of 10 s was chosen to provide 10 scans of each compound transition (both quantitation and confirmation) at a dwell time of 1 ms.

3.1.2. LC-MS/MS analysis

All separation experiments were performed with an Ultimate 3000RS HPLC system from Thermo Scientific (San Jose, CA, USA) outfitted with an ARC-18 LC column (2.7 μm, 100 mm, 2.1 mm) provided by Restek Corporation. Separation conditions, gradient details, and mass spectrometry ESI parameters are available in Tables S2 and S3.

3.1.3. Analytical figures of merit

Calibration curves for all experiments were obtained in the range 0.01 to 100 $ng{\cdot}mL^{-1}$. Four validation points at 0.8, 4, 40, and 80 $ng{\cdot}mL^{-1}$ were used to quantify precision and accuracy. Limits of quantitation (LOQ) were designated as the lowest calibration point with precision values across replicates (n = 4) lower than 20%. Analytical validation and performance criteria were followed based on EU SANTE/12682/2019 guidelines, namely linearity (deviation of back-calculated concentration from true concentration +/- 20%), precision (RSD ≤ 20%), and accuracy (70–120%).

4. Results and discussion

4.1. Importance of standard-internal standard pairing

The deuterated isotopologue is a hallmark of quantitative ambient mass spectrometric analysis, often used at a ratio to target compounds of approaching 1 (Su et al., 2013; Tascon et al., 2017). However, the use of an internal standard per target compound for hundreds of compounds is economically, instrumentally, and practically unfeasible. One of the goals of the current work was to explore the usage of a small number of chemically-diverse internal standards for multiresidue ambient MS quantitation. For the analysis of a multiresidue panel of pesticides in fruit matrices via CBS-MS/MS, internal standard-target compound matching is paramount. In this study, target compounds were matched with internal standards *a posteriori* with a panel of internal standards (n = 13) added to the homogenized sample before dilution and extraction. Matching of internal standards was completed by comparing squared correlation coefficients (R^2) across the linear range tested, with the internal standard generating the highest observed value selected for correction and further quantitation. The process was repeated for LC-MS/MS analysis. Potential sources of poor correction stem from misrepresentative IS behaviour during the extraction, desorption, or ionization processes, or from potential matrix-sourced interferences sharing the same SRM transition as the IS. There is room for additional IS optimization, specifically concerning the concentration of IS per sample. Ideally, the absolute signal observed from the IS should be comparable to the signal observed from the analyte of interest. Due to the diverse panel of analytes and their varying ionization qualities this fine-tuning was not explored, and all IS were spiked at one concentration for all matrices. We sought to demonstrate that this internal-standard pairing approach can correct for matrix effects originating from both the extraction and ionization steps, allowing for comparable quantitative results (± 20%) between direct-to-MS and LC-MS methodologies as discussed in section 3.4 (Beach & Gabryelski, 2013).

4.2. Advantages of sample dilution

Dilution of complex, particulate-containing samples for SPME provides several benefits. From an extraction perspective, dilution of the sample can reduce the proportion of particulate surface area to water, reducing the proportion of analyte bound to matrix components and subsequently increasing the amount of analyte extracted (Alam & Pawliszyn, 2018; Souza-Silva & Pawliszyn, 2015). Increasing the mass of analytes in free-form (*i.e.* amenable to SPME) is important in rapid analysis and in cases where analyte desorption from the matrix is slow. Simultaneously, the increased dilution of a sample (*e.g.* fruit homogenate) results in the use of substantially less sample material, standard, and internal standard use per analysis. In the case of fruit homogenate, dilution also enabled practicality improvements in sample handling (*i.e.* volumetric sample distribution when compared to mass-based sample distribution). Pesticide sorption to organic matter is well described for pesticide-soil systems and correlated with the octanol–water partition coefficient (Sabljić, Güsten, Verhaar, & Hermens, 1995). Thus, an analyte-matrix binding component negatively impacting amount extracted was expected due to the polarity range of the compounds under study (*i.e.* logP −1.2 [thiamethoxam] to 5.9 [etoxazole]) and an investigation of the impact of dilution on the extraction of the pesticide panel was pursued. Signal-to-noise ratios (S/N) and absolute intensity at various fruit homogenate dilution levels were compared and three relationships were observed (Fig. 2), a reduction of S/N with sample dilution, constant S/N with sample dilution, and increasing S/N with sample dilution.

The S/N and dilution relationships observed can be attributed to hydrophobicity differences, with more hydrophilic compounds (logP < 2) expected to have reduced matrix binding and be more negatively impacted by dilution, as observed in neonicotinoids and polar organophosphorus compounds. Mid-polarity compounds (2 < logP < 4) displayed a mixture of behaviour with dilution. However, in cases of reduced S/N with dilution, the observed reduction was less than the dilution factor. Finally, more hydrophobic compounds (4 < logP) displayed increased S/N values with dilution, likely due to high particulate binding and slow desorption kinetics, with dilution increasing the amounts of such compounds available in their free form. It is worth mentioning ionization suppression as a confounding factor. In less dilute samples, the potential increased extraction of matrix-endogenous compounds can result in ESI signal suppression (and thus S/N suppression) and contribute to the apparent effects of matrix-analyte binding. Separation of the confounding variables could be done with a replicate dilution experiment incorporating a chromatographic separation step to reduce or remove the impact of matrix co-extractive ionization suppression. Although not the optimal course of action for all compounds of interest—significant sacrifice was made in S/N values for neonicotinoids and select polar organophosphorus compounds as an example—the methodology improvements were deemed to outweigh the reduced S/N performance observed. Improved or unchanged S/N with dilution was observed for most compounds. This, along with the reduction of fruit homogenate and standards used per sample with the a high dilution level enabling volumetric sample handling, justified the compromise to pursue the 0.1 dilution level for further method development.

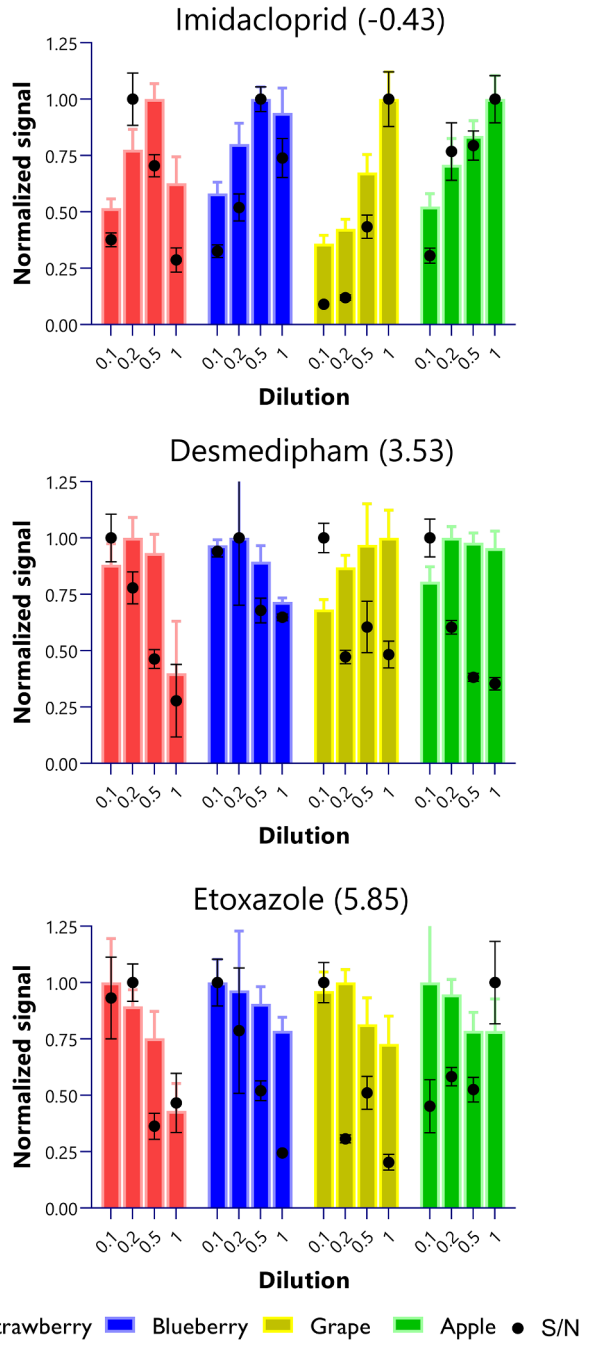

Fig. 2. Investigation of fruit homogenate dilution on signal intensity and S/N ratio given in normalized terms to maximum value (assigned 1.00) and summary of analyte behaviour observed in the compound data set. Dilution levels correspond to 1 g of homogenate spiked at 50 ng/g diluted with 9 mL of water (0.1), 4 mL of water (0.2), 1 mL of water (0.5), and no water added (1). Plots are arranged in increasing logP value, with imidacloprid displaying trends of reduction of S/N and absolute signal intensity with dilution, desmedipham displaying constant/increasing S/N with dilution, and etoxazole displaying increasing S/N and intensity with dilution.

4.3. Coated blade spray as a rapid sample screening and quantitation tool

Upon selection of dilution level, a method comparison of both instrumental approaches was carried out with respect to analytical figures of merit. As shown in Tables 1 and 2, differences in figures of merit are marginal for strawberry, with deviations in LOQs within 1 calibration level. Additionally, this comparison of method performance using both direct coupling and a separation technique allowed for a more robust determination of quantifiable candidates within the concentration range tested. As an example, propiconazole ($C_{15}H_{17}Cl_2N_3O_2$, 341.068794 Da) can generate signal in an SRM channel of prothioconazole ($C_{14}H_{15}Cl_2N_3OS$, 343.031281 Da) for the commonly monitored transition of 344 → 189. Propiconazole has a low-contributing 187 Da fragment, which when coupled with the isotopic distribution of the compound, results in cross-talk in the same channel as prothioconazole. This could result in a false positive if only one transition is monitored using a direct-to-MS technique, highlighting the importance of dual transition monitoring in the workflow presented. The differentiation of

Table 1
Abridged figures of merit for compounds found in strawberry samples via CBS-MS/MS and LC-MS/MS.

Compound	Method	Internal Standard	R^2	LOQ (ng/g)	Accuracy (%, n = 4)				Precision (%, n = 4)			
					0.8 ng/g	4 ng/g	40 ng/g	80 ng/g	0.8 ng/g	4 ng/g	40 ng/g	80 ng/g
acetamiprid	CBS	dimethoate-d_6	0.9904	2.5		104.8	93.3	92.4		4.3	15.5	10.1
	LC	dimethoate-d_6	0.9923	1		101.0	97.6	102.3		8.2	8.2	12.0
azoxystrobin	CBS	malathion-d_6	0.9969	0.5	110.8	102.0	102.0	95.2	8.1	6.4	7.9	3.2
	LC	atrazine-d_5	0.9960	0.5	96.7	94.3	88.3	88.6	15.7	4.6	4.3	6.8
boscalid	CBS	atrazine-d_5	0.9857	2.5		106.2	105.0	86.5		4.8	12.9	3.2
	LC	atrazine-d_5	0.9947	2.5		93.9	92.8	93.2		9.3	9.5	6.2
chlorantraniliprole	CBS	malathion-d_6	0.9940	2.5		106.6	84.1	90.0		15.1	7.3	6.4
	LC	dimethoate-d_6	0.9939	1		94.8	92.6	91.8		20.6	8.3	9.9
cyprodinil	CBS	malathion-d_6	0.9887	5			96.3	99.8			6.6	2.7
	LC	cyprodinil-d_5	0.9935	2.5		109.6	115.0	104.1		12.9	10.3	10.3
difenoconazole	CBS	malathion-d_6	0.9866	2.5		103.8	94.4	93.5		25.0	21.1	13.8
	LC	trifloxystrobin-d_6	0.9913	1		104.8	107.3	112.4		16.9	12.1	13.8
imidacloprid	CBS	carbofuran-d_3	0.9934	1		97.6	86.3	92.6		12.8	16.3	8.7
	LC	dimethoate-d_6	0.9911	5			93.5	97.6			5.0	8.9
metalaxyl	CBS	metalaxyl-d_6	0.9902	0.5	97.1	121.4	113.1	88.9	6.0	13.9	12.3	6.1
	LC	metalaxyl-d_6	0.9979	0.5	104.7	99.3	98.8	99.5	16.0	4.8	3.2	4.2
mevinphos	CBS	dimethoate-d_6	0.9906	2.5		118.0	100.2	93.9		5.8	11.2	8.9
	LC	carbofuran-d_3	0.9948	2.5		97.3	95.6	95.4		12.2	4.5	8.2
myclobutanil	CBS	atrazine-d_5	0.9921	2.5		98.3	106.2	91.0		10.4	8.0	8.3
	LC	atrazine-d_5	0.9937	1		96.6	106.7	101.2		7.5	4.1	6.0
propiconazole	CBS	atrazine-d_5	0.9856	1		97.7	97.4	89.8		20.1	10.6	11.5
	LC	atrazine-d_5	0.9935	2.5		100.6	93.7	94.7		14.0	4.5	3.2
pyraclostrobin	CBS	malathion-d_6	0.9822	2.5		105.2	85.8	95.0		10.0	13.5	10.5
	LC	trifloxystrobin-d_6	0.9877	5			102.6	99.3			10.1	7.5
pyrimethanil	CBS	malathion-d_6	0.9853	2.5		105.5	87.9	98.0		15.0	12.0	4.9
	LC	atrazine-d_5	0.9932	1		96.6	95.4	95.7		3.1	4.3	4.6
quinoxyfen	CBS	malathion-d_6	0.9759	10			90.8	93.3			13.1	12.3
	LC	trifloxystrobin-d_6	0.9821	10			116.0	95.4			12.9	19.3

the compounds in question is made trivial in the LC-MS/MS approach due to their separation characteristics. The comparison of instrumental approaches yielded good analytical figures of merit for all compounds across all matrices tested, with full compound characterization available in Tables E1-E8. Another distinction between instrumental methods is the economy of time and consumables, as the removal of the separation step reduced the analysis time 9-fold and solvent usage 460-fold.

4.4. Analysis of a panel of real samples with LC-MS/MS confirmation

Across the 4 matrices under investigation, 15 real-world samples were analyzed. Within the 3 apple, 3 blueberry, 5 grape, and 4 strawberry samples, a total of 45 pesticide residues were quantified and confirmed within the linear range tested, with an additional 12 residues detected outside of the linear range (> 100 ng/g). Notably, a locally sourced strawberry sample contained the largest number of individual pesticide residues at 13, although all residues complied with MRLs designated by Health Canada. However, one of the detected compounds, propiconazole, was quantified at a concentration above the EU MRL of 50 ng/g at 51.7 ± 7.2 and 51.1 ± 3.6 ng/g via CBS-MS/MS and LC-MS/MS, respectively. In general, good agreement was found between samples analyzed by CBS-MS/MS and LC-MS/MS with Bland-Altman plot analysis for pesticides quantified above 10 ng/g (level chosen as a low concentration cut-off to not bias 95% CI due to inversely proportional percent differences with concentration) resulting in a bias of 2.307 with 95% CI −30.86 to 35.47%, as shown in Fig. 3 (expanded real sample data in Table S5). The results on comparability of methodologies with this small sample set broadly distributed over the compounds under study suggests that the internal standards

Table 2
Comparison of residues found in real-world strawberry samples by LC-MS/MS and CBS-MS/MS

Compound	Sample 1 (ng/g)		Sample 2 (ng/g)		Sample 3 (ng/g)		Sample 4 (ng/g)	
	CBS ± SD	LC ± SD	CBS ± SD	LC ± SD	CBS ± SD	LC ± SD	CBS ± SD	LC ± SD
acetamiprid			573.5 ± 41.7	606.9 ± 44				
azoxystrobin			80.8 ± 6.4	95.7 ± 3.2				
boscalid	13.2 ± 1.6	8.2 ± 0.8	5.2 ± 0.8	2.9 ± 0.3	7.1 ± 1.9	4.2 ± 0.3	3.4 ± 0.7	2.6 ± 0.2
chlorantraniliprole	3.6 ± 0.2	4.4 ± 1			3.7 ± 0.9	3.7 ± 0.4	3.1 ± 0.4	4.3 ± 0.1
cyprodinil			346.7 ± 24.7	485.9 ± 52.1			391.8 ± 29.8	537 ± 39.3
difenoconazole			97.9 ± 14	108.3 ± 6.2				
fludioxonil[Y]			N/A	291.3 ± 18.4			N/A	270.3 ± 20.4
imidacloprid			5.7 ± 0.5	6.3 ± 1.3				
metalaxyl	1.3 ± 0.2	1.2 ± 0.1					13.5 ± 2.5	14.4 ± 0.7
mevinphos			6.2 ± 0.1	8 ± 0.7				
myclobutanil			18.6 ± 1.8	20.2 ± 0.7			2.7 ± 0.7	1.5 ± 0.1
propiconazole			51.7 ± 7.2	51.1 ± 3.6				
pyraclostrobin	4.4 ± 0.9	3.7 ± 1	770 ± 63	849.8 ± 45.9	4.1 ± 2.1	2.5 ± 0.3	4.9 ± 0.8	5.3 ± 2
pyrimethanil	140.5 ± 4.1	162 ± 9			11.7 ± 2.1	13.8 ± 1.1		
quinoxyfen			186.4 ± 18.8	161 ± 7.6				
spinetoram			15.2 ± 1.3	14.4 ± 1.2				

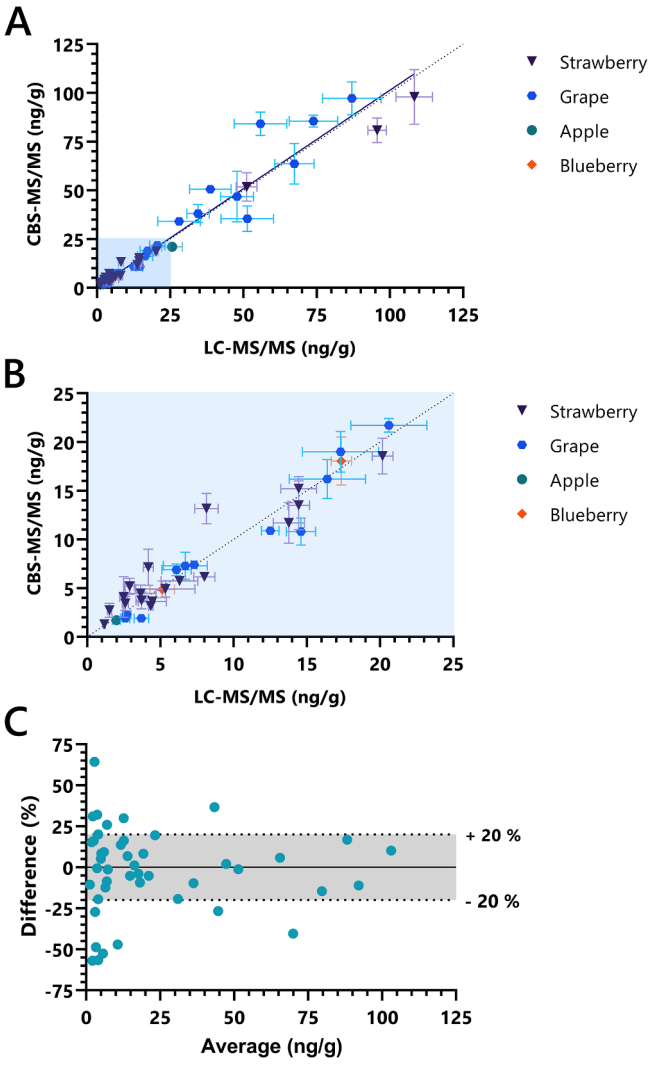

Fig. 3. Summary of all real-world sample quantitation of pesticides (n = 45) with both CBS-MS/MS and LC-MS/MS marked by matrix and by concentration range with the full 0–100 ng/g in panel A, with an enlarged 0–25 ng/g in panel B (1:1 line displayed as a dotted line). Acceptable agreement was observed between instrumental approaches, with a slope of 1.011 (95% CI: 0.9692 to 1.052), intercept of 0.2855 (95% CI: −0.1949 to 0.7659), R^2 of 0.9291. Percent differences (from the average of two results) are displayed in panel C between the concentrations of 0–100 ng/g with full numerical data presented in Table S5.

selected correct for hypothesized ionization suppression differences between LC-MS/MS and CBS-MS/MS, with the former yielding reduced co-elution of matrix extractives and compounds of interest. Further expansion of the sample size using the methodology presented would be desired to form more robust conclusions on method comparability. Pesticide product formulations contain surfactants and stabilizers to improve product application and lifespan in-field (Knowles, 2008; Wang, Li, Zhang, Dong, & Eastoe, 2007). These excipients have been shown to contribute to ESI suppression of ambient mass spectrometric analysis when compared to a pesticide standards (Mainero Rocca et al., 2017). The good agreement demonstrated between instrumental real-world sample results inspires confidence in the correction of these effects via internal standard selection or integrated sample preparation via SPME, or both. Additional support for the validity of real-world sample quantitation can be found upon comparison of ratios of pesticides in commercially available pesticide products. For example, one grape sample was found to contain boscalid and pyraclostrobin at a ratio of 1.86 ± 0.208 and one strawberry sample was found to contain cyprodinil and fludioxonil at a ratio of 1.67 ± 0.251, closely matching the ratios found in boscalid/pyraclostrobin (found at a 1.96875 ratio) and cyprodinil/fludioxonil (found at a 1.5 ratio) commercial formulations (BASF Corporation, 2020; Syngenta, 2020).

4.5. Problematic compound classes and future directions

The methodology presented is not a panacea for the extraction of all compound classes. Of the 204 pesticides investigated in the preliminary method developmental steps, several pesticide classes remained difficult to quantify at the MRLs required by regulatory bodies. In general, difficulties are expected with very hydrophilic (logP > -1) and very hydrophobic (logP > 5) analyte classes. Low extraction of polar compounds is expected as the aqueous matrix effectively competes for polar analytes. On the other hand, poor solubility of very hydrophobic compounds and their strong association with the matrix leads to slow desorption kinetics, resulting in slow mass transfer to the extraction phase. In this study, polar organophosphates such as acephate and methampidphos were unable to be quantified below EU MRLs. However, implementation of lower sample dilution levels, zirconia-based extraction phases to increase selectivity, and/or DART as a ionization technique remain avenues to be explored (Gómez-Ríos et al., 2017; Liu et al., 2020). On the opposite side of the polarity spectrum, the increasing popularity of biopesticides of the avermectin and spinosyn classes and their synthetic analogues present a challenge for the method described due to their suspected substantial matrix-binding (Crouse et al., 2001; Dionisio & Rath, 2016). Addition of organic solvents to reduce the proportion of bound analytes has been demonstrated for hydrophobic analytes in biological matrices (Gómez-Ríos et al., 2018), and was pursued to investigate its impact on the broad range of compounds in blueberry matrix, with 10-fold increases in S/N observed for these hydrophobic molecules. However, such increases came at the expense of the S/N of more hydrophilic analytes due to the simultaneous reduction of affinity to the extraction phase with the addition of acetonitrile (elaborated on in Fig. S1 and Table S6). Such analyte-matrix binding difficulties have been reported for the SPME of pyrethroids from grape homogenate (Souza-Silva & Pawliszyn, 2015). These underperforming compound classes remain the compromise of the method presented, and the largest area of improvement to be further explored in future work through investigation of different sorbents, organic matrix modifiers, and reducing analyte-matrix partitioning with temperature. Since the presented CBS method yields a fast overall time of analysis, repeated extraction of the same sample after addition of small amounts of an appropriate solvent to facilitate mobilization of the hydrophobic compounds can be further explored as a possible solution.

5. Conclusions and future perspectives

CBS-MS/MS was demonstrated as a complementary technique to LC-MS/MS analysis for the quantitation of a multiresidue panel of pesticides in apple, blueberry, grape, and strawberry matrices. The presented workflow significantly reduces analysis time, amount of sample required, and laboratory footprint compared to regulatory gold standards, while providing the analyst with freedom to further increase throughput with direct-to-MS coupling or increase analytical confidence via LC-MS/MS coupling for confirmation with the same sample preparation methodology. Noting this, a direct comparison study between gold standard sample preparation approaches (such as QuEChERS, SE) and the methodology presented is of interest to determine differences more concretely in analysis speed, cost, and efficacy. For the majority of the studied compounds (126 pesticides in apples, 139 pesticides in blueberries, 136 pesticides in grapes, and 135 pesticides in strawberries), analytical figures of merit were found to meet EU SANTE/12682/2019 regulatory standards in terms of linearity, accuracy, and precision. Encouragingly, real-world sample

analysis yielded comparable results (percent differences < 20% for 73% residues) between direct-to-MS and LC-MS/MS approaches, with pesticide ratios found to resemble commercially available formulations closely. Compromises for multiresidue method development in certain pesticide classes, notably the highly polar and non-polar (*e.g.* avermectins, polar organophosphates), were discussed, with sample preparation strategies regarding organic solvent dilution, ionization method, and extraction phase exploration presented as future avenues of research. Additionally, future LC-MS/MS-side optimization could be explored, such as desorption time and solvent optimization, to yield a faster LC confirmation complement.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127815.

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